

1 The Health Benefits of Bioactive Compounds from Milk and Dairy Products

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1.1 The importance of milk and dairy products

Milk and dairy products have been consumed for thousands of years as a wholesome staple diet in many cultures around the world. They provide the necessary nutritional, functional and physiological values to maintain health and wellbeing of the human body. Their nutritional value, due to the presence of the necessary macro and micronutrients for a healthy life style, has been known of for some time. The nutrients from milk and dairy products include proteins and peptides with their complete range of all the essential amino acids. Milk and dairy products also provide a number of physiological properties: the fat and the presence of both saturated and some unsaturated fatty acids, with the latter shown to have an important physiological role such as with the conjugated linoleic acid (CLA); lactose and other minor oligosaccharides; the major and essential minerals such as calcium, magnesium as well as phosphorous; and vitamins, especially the fat-soluble vitamins A, D, E and K as well as some of the water-soluble vitamins such as B12, riboflavin and C. Milk is the only source of nourishment for the neonates as nutritious food for adults. These nutrients provide the necessary elements for growth and maintenance of the human body. Infants can thrive just by consuming milk for several months, obtaining all the necessary nutrients for growth. Milk also provides the additional physiological properties of promoting the health of the baby and protecting against diseases through its immunoglobulins and other whey proteins.

Based on all these facts, milk and dairy products are considered an important component of functional foods and have seen an increase in their production in the last few decades. In this chapter the role of milk and dairy products, their nutritional importance and their role within the functional food sector are briefly described. Subsequent chapters provide a more detailed explanation of the role of the individual components.

Apart from their health benefits, dairy products are also recognised economically as an agricultural commodity and an essential commercial driver, especially in industrialised developed countries. The dairy industry is an important sector within the food industry, the most dynamic and with the highest gross output. Although the number of independent small dairy farms in most developed countries is in continuous decline due to economic pressure and international regulations, the dairy cooperatives and larger farms are on the increase

and are able to reduce the overall cost of unit production and prosper in a demanding and competitive market.

The dairy industry, including the primary producers, has been at the frontline of innovation and diversification with emphasis on continuous progress to include animal breeding, quality of feed, new technologies and investment in research and development. Consequently, the development of new products with added value and the number of different varieties of dairy products have also been experiencing an increasing trend, especially the functional products which claim to improve the overall human health and wellbeing. Recently however, most research has focused on the additional health benefits provided by milk, its fermented products and the individual fractions, whether from the protein and its peptides, the fatty acids, the sugars, the minerals or the vitamins.

The importance of dairy products as part of a healthy daily dietary intake has also been emphasised by many government and other scientific bodies, not only for their economic value but also for their nutritional significance. This was clearly emphasised by the healthy balanced diet promoted by the Food Standards Agency (FSA 2007) and the nutrients of concern advocated by the United States Department of Agriculture (USDA 2005). However, it was found from the UK National Diet and Nutrition Surveys (Gregory 2000) that energy intake from fat consumption was almost 50% of total energy consumed; this is far greater than the recommended level, that of 30%. Prior to this recommendation, the dairy industry has been actively involved in introducing new products to satisfy the consumer demand for healthier products, especially those of low-fat (e.g. skimmed and semi-skimmed milk) and high-protein content.

1.2 Dairy products: Concerns and challenges

Although there have been numerous published research data indicating the positive role and benefits of consumption of milk and dairy products to human health, in the last few decades there has also been much adverse publicity regarding the major components of milk and dairy products: fat, protein and lactose. Concern has been raised regarding these three components as being either linked to the high fat content and saturated fatty acids level, protein allergy or lactose intolerance and any adverse symptoms associated with the consumption of these products. Although these three components do contribute to some undesirable side-effects, it is usually only a small segment of the population who are affected. It is however unfortunate that such unfavourable publicity has influenced a larger sector of the society, composed of normal healthy individuals with no adverse reaction to any of these major constituents in milk. Although some people have a genuine need to replace milk with other sources of protein such as soya, a bias against the consumption of milk should be based on real scientific facts and weighed up against the health benefits of consuming milk and dairy products.

Public misconceptions regarding the consumption of milk and dairy products must be addressed to restore the confidence of the public in the health benefits of milk and dairy products. There are huge challenges ahead facing all those concerned about the health and welfare of society – scientists, technologists, health practitioners, marketers and probably psychologists – in tackling this problem and changing consumers' concern or fear of dairy products, which is mostly based on saturated fat, protein, allergy and lactose intolerance. In tackling hostility towards milk, scientists and technologists have identified and explored methods to

eliminate or minimise some of these adverse reactions through physical or chemical means. For example, although fat reduction in milk is still a controversial issue, this has been achieved by separating fat by centrifuging and introducing skimmed, semi-skimmed milk and low- to very-low-fat dairy products. Lactose content has also been reduced by physical means using membrane technology such as ultrafiltration. On the other hand, chemical means have been used to reduce or modify the protein content by partial enzymatic hydrolysis using proteolytic enzymes to produce a hypoallergenic milk powder especially for infant formula. However, the disadvantage of protein hydrolysis is the possibility of producing some bitter peptides which require further debittering processes (Minagawa *et al.* 1989; Gallagher *et al.* 1993, 1994). Removal of lactose can be achieved by its complete conversion to galactose and glucose by enzymatic hydrolysis using β -galactosidase or lactase. However, lactose hydrolysis increases the sweetness of milk, which might be unacceptable to those familiar with the low sweetness of milk. Nonetheless, this would be beneficial in fermented products.

The complete removal or reduction of the fat from milk might not be that challenging technologically, but the sensory quality is adversely affected and the resulting milk tastes quite watery and lacking in the body associated with full-fat milk. Fat reduction in dairy products also affects the texture and other functional properties, compensated for by the use of additives in some dairy products to improve consistency and texture. The nutritional quality of fat-soluble vitamins is also compromised, and external sources are needed to replace what has been removed to keep a reasonable amount of these important vitamins. Fat removal also reduces the concentration of the conjugated fatty acids (CLA), a vital component in preventing many diseases such as Type 2 diabetes, inflammation and cancer (Zulet *et al.* 2005). A comprehensive description of the importance of CLA in human diet with a wide range of examples is given in Chapter 6.

With all this negative and adverse publicity regarding the consumption of milk and dairy products, the only way to counteract this and increase their consumption is to develop new products in a similar trend to that of the emerging functional foods movement. The use of milk or the separation of certain ingredients from milk has also been at the frontline of the introduction and promotion of many dairy products ranging from probiotic fermented milk, colostrum, whey protein concentrates/isolates and bioactive peptides from the hydrolysis of casein and whey proteins. Some of these ingredients have been used as nutraceuticals and have, to some degree, compensated for the loss in revenue for some sectors within the dairy industry.

1.3 Dairy products and public health

The function of public health in any country is to protect the health and well-being of the people by preventing disease and ill health and promoting a healthy-living lifestyle. Diseases such as coronary heart disease (CHD), cardiovascular disease (CVD) and cancer and conditions such as diabetes, obesity and mental ill-health are a major concern and have seen an alarming surge in reported incidences. To maintain and improve public health, it is essential to provide the necessary information and tools to the public and make them aware of their role and responsibilities in achieving a healthier life. This could include quality of diet and associated nutrients as well as other important factors such as physical activity, education, environmental pollution and stress, which all have a role to play in overall welfare.

The concern about public health and the cost of healthcare is a major driver for many countries, especially in the western industrialised world. Expenditure on public health is mounting and in continuous increase year after year in most countries. In the UK, net expenditure is huge and has increased from £49 billion in 2001/02 to £104 billion in 2010/11, with mental health having the highest share. This total expenditure represents almost 8% of the net expenditure as a proportion of the gross domestic product (GDP; NHS 2011), with similar trends seen in other developed countries. The rise in the population of the elderly due to advances in medicine, nutrition and care provided by society has also added extra expenditure. In this section only one public health concerns is discussed.

1.3.1 Heart disease

Heart disease is a major cause of death, especially in the western world, and is the second-highest cost of health to the NHS in the UK after mental health (NHS 2011). It is well known that diet and lifestyle greatly contribute to the onset and the development of the disease, which could be easily managed. One of the important markers of heart disease is arterial stiffness. In recently published research, Crichton *et al.* (2012) investigated whether the consumption of dairy products could have any effect on the arterial stiffness. The study used pulse wave velocity (PWV) as an independent predictor of mortality due to cardiovascular damage. Their results demonstrated a negative linear relationship between the consumption of dairy products and the pulse wave velocity, independent of nutritional, demographic variables and other cardiovascular disease risk. Interestingly, they recommended further research studies to determine whether the consumption of dairy products would reduce arterial stiffness and cardiovascular disease risk in the elderly.

In a similar study to investigate the relationship between the consumption of dairy products and aortic PWV and arterial stiffness as a predictor of cardiovascular disease and mortality, Livingstone *et al.* (2012) used data from the Caerphilly Prospective Study. They concluded that consuming dairy products (excluding butter) does not adversely affect the arterial stiffness and metabolic markers.

In association within the Caerphilly Prospective Study, Elwood *et al.* (2007) examined the relationship between the consumption of dairy products and metabolic syndrome. The collected data from the metabolic syndrome were based on body mass index (BMI), blood pressure, high-density lipoprotein (HDL), the fasting blood glucose and insulin and fasting plasma triacylglycerol. Their investigation demonstrated a negative relationship between metabolic syndrome and the consumption of milk and dairy products, and no significant relationship between the incidence of diabetes and the consumption of dairy products.

Based on the research data described above, milk and dairy products should not be considered as unhealthy or the source of heart disease. However, further research is required to establish the link between diet and heart disease.

The health debate regarding the link between the consumption of saturated fatty acids and CVD, CHD and stroke, in isolation from the factors which cause heart disease, needs to be tackled in a more objective way based on more epidemiological studies rather than relying on existing data or using univalent analysis. The role of milk and dairy products should not be undermined considering that they provide protein, peptides, CLA, fat-soluble vitamins

and important minerals such as calcium, potassium and zinc. All these could protect against many diseases including heart disease, diabetes, osteoporosis and stroke. Many long-term epidemiological studies involving a high number of subjects concluded that there is no direct link between consumption of a low-fat diet and a longer life expectancy, or whether a reduction in fat intake and its replacement with fruits and vegetables will reduce the incidence of CHD, CVD or stroke (Taubes 2001; Howard *et al.* 2006; Siri-Tarino *et al.* 2010).

It should be emphasised that milk and dairy products are a vital constituent of daily diet and consumers should be given a fair and impartial view about the importance of consuming a balanced diet. Indulgence in consuming any food can be unhealthy, which is true for many other food ingredients or additives. The challenge is to maintain both a healthy balanced diet and lifestyle.

1.4 Major nutrients in milk

The major constituent in milk which contributes towards improving human health includes protein and its bioactive peptides, fat and lactose and its derivatives. Each of these are discussed briefly in the following sections; for more details see Chapters 2–8.

1.4.1 Proteins

Caseins and whey proteins are present in milk at a concentration of 3.3–3.5% and have important nutritional value as well as many physiological functions after digestion. Milk proteins are important for the growth and maintenance of the body as well as providing bioactive peptides (BAP) and essential amino acids after their hydrolysis. Bioactive peptides derived from milk protein hydrolysis have an array of bioactive properties such as antihypercholesterolemic, hypotensive, anticarcinogenic and immunomodulators. More coverage is given in the sections below and in Chapters 2, 4, 7 and 8.

It appears that BAPs derived from milk protein offer better health benefits compared to other types of proteins such as soy protein, especially in building up skeletal protein. It has been shown that milk-derived peptides stimulate the uptake of amino acids after resistance exercise, consequently giving a larger increase in lean muscle which is beneficial to young athletes as well as strengthening muscles in the elderly (Phillips *et al.* 2005). Chapter 5 describes the importance of dairy products in sport nutrition.

Some whey proteins exhibit bioactivity without hydrolysis, such as immunoglobulins and lactoferrin. The immunoglobulins are considered the primary defence mechanism against any pathogenic micro-organisms in the gastrointestinal tract (GIT) of the infant, which is not yet fully developed to prevent infection. The immunoglobulins constitute the major protein in colostrum, which is the secreted milk by the mother postpartum. The highest concentration occurs in the first few hours at about 20% and drops to 0.5% after 48–72 hours, remaining at that level throughout the lactation period. Chapter 3 describes the health benefits of colostrum. Bovine colostrum and whey protein concentrate and isolates are available on the market for their health benefits, especially in modulating the immune system and muscle growth which is why they are mainly used by athletes (Phillips *et al.* 2005).

1.4.2 Milk fat

Milk fat provides energy, the essential fat-soluble vitamins A, D, E and K, some of the essential fatty acids including oleic acid and the conjugated linoleic acid (CLA) and its several isomers. These have all been studied by many scientists for their physiological functions such as anticancer and anti-atherogenic properties (Lock & Bauman 2004; Bauman *et al.* 2006). Of the isomers, *cis-9* and *trans-11*-octadecadienoic acid are prepredominantly found in milk fat in high concentration. They are mainly produced by anaerobic fermentation of bacteria in the rumen via the biohydrogenation of the polyunsaturated fatty acids. Some studies have linked CLA to its function as mutagenesis inhibitor and anticarcinogen (NRC 1996; Park *et al.* 2001), which might also be associated with its antioxidant property.

Although milk fat contains some saturated fatty acids like many other foods that does not mean that milk or dairy products are unhealthy products and should be avoided at all cost. A reduction in the consumption of saturated fatty acids (SFA) has been promoted by governments and several researchers in the last few decades. The link between milk fat and its saturated fatty acids and chronic heart disease has been under debate for several decades. Although many researchers have considered SFAs as one of the contributory factors in heart disease, so far there has been no real study or confirmed data to demonstrate conclusively a direct link between cardiovascular disease (CVD) and milk fat or to implicate dairy products in heart disease. Some useful data could be gathered from meta-analysis in order to provide a more comprehensive picture on this subject. Although there has been a lot of emphasis on reducing the intake of fat, SFAs and refined carbohydrates, the possible connection between obesity (and many other factors) and CVD should also be taken into account.

Although consumers should understand the importance of dietary fat for general health, heart disease is a multi-faceted problem and should not be solely linked to the consumption of SFAs or just a few foods or dietary habits. Other issues such as nutritional education, physical activity, smoking, stress, etc. all play a role in cardiovascular diseases. To emphasise this controversial issue, Taubes (2001) concluded that, after 50 years of research, there was little evidence to link a low consumption of saturated fat to prolonged life. Similarly, a recent meta-analysis of 21 prospective epidemiological studies by Siri-Tarino *et al.* (2010) concluded that there was no significant evidence to associate dietary saturated fat with an increased risk of CHD or CVD. This clearly shows that the relationship between fat, saturated fatty acids, cholesterol and CHD is a more complex matter than initially thought, and the risk of heart disease is multi-faceted (Parodi 2009). The impact of milk fat naturally enriched in *trans* fatty acids (TFA) has recently been examined in human clinical studies. It was found that even when TFA consumption was at the upper range of normal dietary intake, there were no negative effects on plasma biomarkers of CHD risk. It seems that there is little or no epidemiological, clinical or animal-model data indicating that consumption of natural milk fat, as a source of TFA, has a negative impact on human health (Motard-Belanger *et al.* 2008). Another recent study by Petyaev & Bashmakov (2012) highlighted the ongoing debate regarding the 'French Paradox' related to the consumption of dairy products and heart disease. It was observed that in France, despite high consumption of cheese and dairy products, CVD mortality is still low. Although this result was initially linked to the consumption of red

wine and the presence of resveratrol, this link was not apparent in other countries. The link between milk fat and heart disease must therefore be proven beyond any doubt before a firm conclusion is drawn.

1.4.3 Lactose and its derivatives

Bovine milk contains about 4.6–4.8% lactose, which is lower than that found in human milk of about 7%. Lactose and its derivatives provide about 50% of the energy requirements of the infant (Vesa *et al.* 2000). The two components of lactose – glucose and galactose – play an important biological role in the body as the major source of energy supply and for brain development as part of the galactocerebroside (Kunz *et al.* 1999, 2002). In addition to lactose, about 15–18% of the total sugars in milk are present as lactose derivatives in the form of oligosaccharides in straight or branched chains. These vary in length over the range 3–8 monomers and contain mostly galactose, fucose and sialic acid (Fukuda *et al.* 2010).

It is of great interest to discover that human milk contains higher amounts of lactose and its derivatives compared to other mammals such as cows and goats; milk from the latter examples are also lacking in, for example, the fucosylated derivative (Newburg 1996; Urushima *et al.* 2001). It has been indicated that these oligosaccharides have physiological functions expressed mainly in the intestinal tract; they could act as prebiotics for the growth of the beneficial microflora in the gut such as *Bifidobacterium bifidum* which are naturally present in the gut of breast-fed babies, hence improving their general health (Venema 2012). Lactose derivatives have also been considered to improve health through the modulation of the defence system against pathogens, and could act as receptor analogues and prevent pathogenic micro-organism attachment to the epithelial membrane of the colon. Several of these oligosaccharides have also been isolated from Bactrian camel milk and colostrum in order to study their physiological activities (Fukuda *et al.* 2010).

One of the problems associated with milk consumption, experienced by small sector of the population, is lactose intolerance. It is well known that these people feel or perceive the adverse effect when consuming milk or dairy products, even when only a small amount of lactose is in the product such as in fermented milk products. Lactose is usually hydrolysed by the brush border enzyme β -galactosidase to galactose and glucose. With lactose-intolerant individuals, however, the lack of this enzyme leads to lactose fermentation inside the intestinal tract producing different acids (e.g. lactic and acetic) and gases (e.g. carbon dioxide) causing flatulence and discomfort. Lactose intolerance is more apparent in developing countries where milk consumption is usually low and the body produces a much smaller amount of the enzyme. This problem is less likely with fermented dairy products (e.g. yogurt), where the added bacterial culture utilises most of the lactose as the sole source of energy for their growth.

Lactose intolerance has been tackled by the dairy industry by the introduction of products containing low lactose or even lactose-free dairy products. Lactose is either physically removed using membrane technology such as ultrafiltration, or by direct enzymatic hydrolysis as indicated in Section 1.2.

1.4.4 Vitamins and minerals

Minerals in milk play an important role in the physiological functions of the body. It is well known that bioavailability of minerals is usually higher from animal sources, including milk, compared to that from plants. Milk and dairy products are a rich source of dietary calcium necessary for bone formation and, together with phosphate and vitamin D, they contribute to healthy bone structure. The lack of dietary calcium has been linked to osteoporosis for decades, a metabolic bone disease usually associated with a decrease in bone density and increased porosity, fragility and risk of fracture. Although osteoporosis has been linked to factors other than nutrition, such as genetic, environmental and lifestyle, it seems quite likely that calcium is the major factor in keeping and providing a supply of the lost calcium from the bone. Further investigation is however needed to establish the role of vitamin K in maintaining bone density (Vermeer *et al.* 1995; Szulc *et al.* 1996; Booth *et al.* 2000).

The importance of vitamins and their physiological role in improving health through the prevention of many diseases has been known of for a very long time. One of the few controversial issues related to the removal of fat from milk and dairy products is fat-soluble vitamin deficiency such as A, D, E and K. It has been shown that vitamin D has another physiological role regarding the onset of depression in older men and women. In a 6-year study of 531 women and 432 men aged 65 and over, Milaneschi *et al.* (2010) found that there was a link between 25-hydroxyvitamin D deficiency and depression. This was carried out by measuring the vitamin level in the serum and the signs of depression related to levels below 50 nmol L⁻¹. Chapter 9 describes the role of vitamins and minerals in human diet in more detail.

1.5 Dairy products as functional foods

Functional foods have been identified as any food which contains biologically active compounds that provide physiological functions, regardless of their normal nutritional value as a healthy food (Howlett 2008). Physiological functions include antioxidant activity, cholesterol reduction, immune modulation and lowering of blood pressure. Examples of functional foods include food and drink products enriched with vitamins or containing omega-3 fatty acid, probiotics, prebiotics, antioxidants, phytochemicals and many others. The most common and well-known functional ingredient which has also been used in many food formulations, including dairy products, is omega-3 fatty acid, especially the docosahexaenoic acid (DHA) and the eicosapentaenoic acid (EPA) which is mainly sourced from fish oil and, to some degree, from microalgae.

The functionality of these ingredients/foods has been known of for thousands of year throughout the world, especially in the Far East where plant infusions such as tea, herbs and mushrooms were used as a remedy for certain illnesses. The use of yogurt and honey is another example of food used for its tried-and-tested health remedies. The functionality is derived from the bioactive compounds already present in the food, either embedded or as part of one of the constituents within the food which exhibits the functionality only upon their release from the tissue or after hydrolysis e.g. peptides (Hartmann & Meisel 2007; Korhonen & Pihlanto 2007).

Nutraceuticals, a term used to combine nutrition with pharmaceuticals, have recently been recognised as any naturally occurring compounds extracted from food which could be used as supplements, similar to many available supplements in the market such as vitamins and minerals. The three terminologies – functional foods, bioactive compounds and nutraceuticals – have been used interchangeably in this chapter to describe food or some of its constituents for their physiological role in offering health benefits in addition to their normal nutritional value. The less-common name of ‘pharmafood’ has also been introduced to describe some functional foods with more specifically targeted physiological functions.

The consumption of functional foods is associated with wellbeing and health benefits to ameliorate symptoms and remedy or prevent certain diseases. A further development in functional foods was initiated by believing that diseases (especially chronic) cannot be cured just from the use of medication in the long term, but that other traditional and natural remedies are also required. This belief was also instigated by many published data indicating the health benefits of certain foods. However, the use of functional foods is yet to be proven and acknowledged clinically. Although the pharmaceutical industry offers a wide range of medication to cure many illnesses, the trend is shifting towards the use of food and some of its bioactive ingredients as an alternative or complement to medicine. The consumption of certain fruits, vegetables, herbs and dairy products, especially the fermented dairy products, has been recommended as part of a healthy diet to provide essential nutrients and ease or cure many chronic diseases. Fermented dairy products containing probiotic bacteria are now a well-established functional food in most countries.

However, many organisations within Europe such as the European Food Safety Authority (EFSA), the UK’s Food Standard Agency (FSA) and the American Food and Drug Administration (FDA) have not yet fully accepted all the claims presented to them by the food industry; they have however accepted, with some reservation, that a limited number of food and ingredients offer certain functionality. Many functional ingredients have been accepted to be used in food fortification or as supplements such as the omega-3 fatty acid and stanols from plants, non-starch polysaccharides/dietary fibres and many others, some with restrictions on label declarations. In some cases, there has been a heated debate between food manufacturers and legislative organisations regarding whether health claims can be substantiated.

Historically, some foods have long been used for their health benefits and functional properties. The recent increasing interest in functional foods is a result of their acknowledged effect on health, especially regarding heart disease associated with hypertension and cholesterol level in blood. The demand by the consumer for functional foods has been fuelled by the wealth of information, published literature and the continuous marketing and advertising of the food industry regarding the health benefits of functional foods, especially those linked to fermented milk and dairy products. The emphasis on the importance of these products and the different varieties of fermented milk drinks, yogurt, cheese and ice-cream has increased consumer interest; the market is increasing throughout the western world but not yet to the same extent as in Japan, for example. The estimated market value of functional foods in 2008 was \$80 billion (Vergari *et al* 2010) could reach well over \$100 billion with average annual growth of more than 5% per annum. This is true within developed countries as well as in the Far East and China.

Many of these functional foods have not yet been fully recognised, clinically proven or licensed in the western industrialised countries. Although there is no clear legislation to

describe their use, so far they have been covered under other food legislation imposed by individual countries such as the FSA in the UK and the FDA in the US. In Japan, for example, clearer definition and legislation have been established by the Japanese authority where more than 200 functional food products are regulated by Food for Specified Health Use (or FOSHU; Shimizu 2003). In the past several decades, Japan has pioneered and spearheaded a functional food initiative; it is considered to be the largest-consuming country of these products with a well-matured market and willing and appreciative consumers (Arai *et al* 2002).

Some consumers are yet to be assured that functional foods offer the health benefits declared on their label. Such declarations need to be rigorously monitored follow labelling regulations and, where possible, backed by some known human interventions. In the European Union as well as the US and Canada, such declarations are strictly controlled under specific official regulations. Any claims made outside these regulations must be accompanied by a disclaimer indicating that the product is not intended to, for example, prevent or cure certain diseases. Apart from the typical health benefits associated with functional foods, some have also been considered or used as performance enhancers for athletes, immune modulation through gut health improvement, mental enhancement (described as 'brain' food) and relaxing agents (e.g. serotonin).

A tremendous amount of research has been published on the use of nutraceuticals, mostly based on *in vitro* studies. Fewer *in vivo* studies have been carried out on animals and in clinical trials. However, the approval of nutraceuticals by the authorities would be less problematic than that of pharmaceutical compounds as the former are originated from food with limited processing; the latter are associated with more stringent legislation and ethical documentation.

The emphasis in the last few decades has been on firmly establishing milk, dairy products and certain components of milk origin (such as whey protein) as an important segment of functional foods, nutraceuticals and bioactive compounds. Some of these functional foods of milk origin have included fermented milk products with probiotics, whey proteins (whether concentrate, isolate or as nutraceutical materials such as α -lactalbumin, β -lactoglobulin, lactoferrin, lactoperoxidase and other minor constituents) and bioactive peptides from protein hydrolysis and oligonucleotides. Others milk constituents include the fatty acid conjugated linoleic acid (CLA), calcium and lactose derivatives. The list is increasing in number as consumers are becoming more aware of the importance of these ingredients that are either already present in the food or are added to the product. Based on this, milk and its individual components in their intact, modified or hydrolysed forms can be referred to as functional foods and their bioactive constituents as functional ingredients or nutraceuticals. This undoubtedly indicates the importance of milk as a major source of essential nutrients and bioactive compounds for healthy living.

Milk and dairy products are an important sector of functional foods; their individual components such as protein, peptides, fat and fatty acids, sugars, minerals and other minor constituents have been reviewed here as a source of nutrients and provider of physiological functions, reflecting the continuous advancement and progress in this field of science and technology. These findings have been exploited by the industry to produce either nutritional supplements or products that are healthy and tasty. In this context, the emphasis in the following section is on milk and dairy products as functional foods, concentrating mainly on the bioactive peptides derived from total milk proteins as well as the fermented probiotic yogurt

drink and whey proteins. More detailed information is provided in most of the chapters and in Sections 1.6 and 1.7, highlighting the role of each product or component in providing functionality and as bioactive compounds.

The bioactivity and health benefits of some functional dairy products, such as probiotic fermented milk drinks and yogurts, are within the product itself. In some cases, the challenge might arise when functional ingredients are to be added to the product. This is true when a product is to be enriched with pure CLA, which could give an objectionable fishy flavour even when added at lower concentrations. To avoid this problem, manufacturers tend to use the micro- or nano-encapsulated form. This encapsulated form can also offer additional protection from environment processes such as oxidation.

1.6 Bioactive compounds from milk

Some intact proteins could provide physiological functions in addition to their nutritional value. During the digestion of most proteins, some of the released peptides offer additional physiological functions; these so-called 'bioactive peptides' are discussed along with other bioactive compounds in Section 1.6.1 and in more details in Chapters 2–9. The protein in this case is considered as the 'parent' or the precursors of these bioactive peptides which are derived either after digestion, as a result of fermentation or by hydrolysis using any source of proteolytic enzymes. Most of the basic nutrients released by hydrolysis such as amino acids are used as the building blocks for the growth and maintenance of the human body; bioactive peptides, which are needed in minute quantities, offer the additional benefits of protecting the human body from various diseases and dysfunctions of certain organs. The function of these bioactive peptides are similar to that of vitamins and minerals in that they are needed in minute quantities and can provide the body with additional health benefits and protection from certain diseases and malfunctions.

Bioactive compounds have been identified as either naturally occurring endogenous chemical compounds present in foods, such as lactoferrin and immunoglobulin, or produced as a result of the breakdown of the protein in food by the digestive enzymes in the human gastrointestinal tract (GIT). The bioactivities of these peptides are either demonstrated locally, such as antibacterial activity within the GIT, or induce some physiological effect by stimulating the immune system. They can also interact and bind to specific receptors on the outer surface of the epithelial cell membranes and be transported via the bloodstream to reach a specific target organ.

Fermented food can also be a vital contributor to bioactive compounds due to the presence of the beneficial micro-organisms such as the well-known lactic acid bacteria (LAB) or by the production of certain metabolites which provide their own specific health benefits. Most of the bioactive compounds are stable and do not undergo further breakdown; they can be absorbed through the epithelial wall of the GIT and be circulated to all the tissues in the body to provide health benefits and prevent DNA damage. Interest in bioactives or biologically active components (BAC) and their presence in or addition to foods has been growing at a fast rate in the last few decades due to their specific health benefits. Although knowledge of their health benefits has been widely available for some time, it is only recently that methods to isolate and identify these specific compounds have been developed; scientists have been studying these

compounds using modern analytical techniques such as reverse-phase high-performance liquid chromatography (RP-HPLC) and liquid chromatography mass spectrometry (LC-MS), among others. *In vitro* and *in vivo* laboratory experiments on animals as well as several clinical trials have also been conducted to confirm their functionality and safety. Most of the biological activities have been tested *in vitro* adopting different chemical methods or using cell lines as well as *in vivo* and in some clinical trials.

The health benefits and the bioactivities derived from the consumption of milk and dairy products, their individual isolated constituents and derivatives as well as bioactive peptides derived from protein hydrolysis are briefly outlined in the following sections.

1.6.1 Bioactive peptides

During protein hydrolysis by proteolytic enzymes, whether by the digestive enzymes, those produced by the microbial cultures added to milk or a combination of both, the nutritional value of the resulting peptides is associated with the amino acids they contain. In addition to their nutritional value, some of these peptides have been shown to have biological or functional activities which could have physiological properties such as antihypertensive (FitzGerald *et al.* 2004; Hernandez-Ledesma *et al.* 2007; Bader & Ganten 2008). The activities of these peptides are exhibited only when they are released from their original precursor or 'parent' protein, where they exist in a dormant or inactive state. These bioactive peptides can vary in length between 3 and 20 amino acids with one or more physiological effects. Several other macropeptides, up to 100 amino acids in length, have also been identified to have physiological function. It has been shown that the short-length peptides can be absorbed into the bloodstream through a specific peptide transporter mechanism in the epithelial wall of the intestine, and undergo no further hydrolysis by the blood serum peptidases. They eventually reach their particular target organ in the body where they perform a specific function. Large-scale production of such peptides is normally carried out using exogenous enzymes to hydrolyse the protein under certain conditions, followed by extraction and purification. Peptides have also been synthesised with good purity, quality and different chain length on a small- or large-scale operation using liquid- or solid-phase peptide synthesis. Such techniques could synthesise up to 200 amino acid peptides.

It has been shown that conjugation or interaction of some peptides with other molecules such as a simple sugar e.g. glucose, xylose or arabinose or an oligosaccharide could induce bioactivity or even enhance it. For example, it was found that glycation of β -lactoglobulin by attaching arabinose or glucose could increase the antioxidant activity (Chevalier *et al.* 2001). Most of the research on the bioactivity of peptides has been on the antioxidant, hypotensive and hypocholesterolemic effects.

All the above-mentioned physiological activities and functions related to the presence of BAC, whether from peptides or other food constituents, have been assessed either *in vitro* using different chemical assays, using cell lines or a model digestive system. A dynamic gastric model (DGM) has been utilised to investigate the exposure of food to different pH values and enzymes, for example: using different amylases and proteinases at pH 7.0 to resemble the action in the mouth; or using pepsin at pH 2.0 (the condition in the stomach) followed by pH 6.0 using pancreatic juice containing enzymes such as those of proteolytic, lypolytic and

amylolytic nature. *In vivo* assessment, on the other hand, has been based on animal experiments, clinical trials and human interventions. The physiological activities provided by the various bioactive compounds can be summarised as follows.

Antihypertensive/hypotensive properties

The mechanism of hypertension has been linked to the angiotensin-I-converting enzyme (ACE) which is needed for the rennin angiotensin system. The conversion of angiotensin I to angiotensin II causes the restriction of blood flow, hence the increase in blood pressure. It is known that the inhibition of the ACE action help to normalise blood pressure. Some of these bioactive peptides are derived from milk protein and other protein sources as short peptides. These peptides have been shown to lower the blood pressure *in vitro*, *in vivo*, through animal experiments as in spontaneous hypertensive rats (SHR) and in a few clinical trials (Pihlanto-Leppälä *et al.* 1998; Li *et al.* 2004).

The antihypertensive property of the peptides has been studied by many researchers *in vivo* and *in vitro* on crude casein hydrolysates and whey protein digest (Pihlanto-Leppälä *et al.* 2000; FitzGerald *et al.* 2004). The main peptides which have been linked to this property are the short di- and tripeptides such as those having the amino acid sequence of VY, IPP and VPP. These peptides are mainly produced from the hydrolysis of β -casein and κ -casein producing, for example, fraction 84–86 and fraction 74–76, respectively. However, peptides containing 16–19 amino acids have also been shown to induce an antihypertensive property. The production of such short di- and tripeptides can be achieved by enzymatic hydrolysis or as a result of fermentation. In Japan, the fermented sour milk ‘Calpis’ has been marketed as containing the tripeptide IPP and VPP. In Europe, a Finnish company also produces fermented milk called ‘Evolus’ which is marketed as containing ACE inhibitor peptides (Korhonen 2009).

To find out the real effect of these peptides on blood pressure, a meta-analysis of many clinical trials was undertaken by Pripp (2008). In this study, it was concluded that there was a significant reduction in blood pressure when peptides from food proteins were used. In cases of mild to medium hypertension, it was suggested that these peptides could be used as part of a diet, a supplement and/or an alternative to existing pharmaceutical drugs.

The blood-pressure-lowering effect has also been associated with fermented milk products that contain γ -aminobutyric acid (GABA). Inoue *et al.* (2003) showed that feeding fermented milk containing GABA to a group of 39 patients with mild hypertension for 12 weeks lowered the blood pressure significantly within 2–4 weeks.

Antihypercholesterolemic/hypocholesterolemic peptides

Although cholesterol plays an important physiological role in humans, it can also be one of the major factors in heart disease. Its exogenous source is that of food and the rest is produced in the liver. It is present in the blood as free cholesterol or is mostly bound with protein in different forms, the most important forms being the low- and high-density lipoproteins (LDL and HDL). High cholesterol levels in the blood (especially LDL) is one of the factors, among many others, which cause heart disease through atherosclerosis (Stein *et al.* 2005). The pharmaceutical industry has introduced several medications as antihypertensive drugs such as statins which inhibit 3-hydroxy-3-methylglutaryl hydroxymethylglutaryl-CoA

(HMG-CoA) reductase, an enzyme necessary for the formation of endogenous cholesterol. Statins are usually prescribed to people with high cholesterol and at risk from cardiovascular disease.

There are other ways to reduce levels of cholesterol; one of these is through diet including some of the constituents in foods such as plant protein, soluble fibres, plant sterols, probiotics and prebiotics (Taylor & Williams 1998). It has also been shown that some milk protein hydrolysates as well as the small peptides isolated from the hydrolysate, mostly the di- and tripeptides, could also inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase to exhibit a similar effect to that of statins. Several *in vivo* studies on human subjects demonstrated the hypocholesterolemic effect of fermented milk products (Lewis & Burmeister 2005). It has also been reported that tryptic hydrolysate of β -lactoglobulin prevented cholesterol absorption through direct interaction with the cholesterol (Nagaoka *et al.* 2001).

Antioxidants

The oxidation process in the human body is a natural and important metabolic mechanism within the cell which also results in the formation of reactive oxygen species (ROS) as free radicals. When in excess, these free radicals, such as the hydroxyl (OH*) and peroxy (OOR*) radicals, could damage and destroy the cell by affecting the lipids, protein and nucleic acid causing disease and pathogenesis. The damage and the apoptosis of the cells could have a major role in the manifestation of some diseases such as Alzheimer's disease, atherosclerosis and inflammation. The body has its own defence system to counteract the effect of these free radicals, by stabilising them through its own endogenous antioxidant system through catalase, glutathione peroxidase and superoxide dismutase. A similar stabilising effect could also take place by other non-enzymatic actions such as: ascorbic acid and α -tocopherol; by some bioactive peptides such as caseinophosphopeptides (Diaz & Decker 2005; Kim *et al.* 2007) or those derived from the hydrolysates of e.g. β -lactoglobulin (Hernandez-Ledesma *et al.* 2007), glutathione and carnosine (Babizhayev *et al.* 1994); and by the presence of some amino acids such as cysteine, histidine and tyrosine.

In some cases, oxidative stress could take place when natural endogenous antioxidants cannot cope with the surge of free radicals as a result of intensive exercise by endurance athletes or any other reason. During any imbalance due to the high level of free radicals and low level of endogenous or exogenous antioxidants, pathogenesis of some diseases will materialise, especially in vulnerable groups such as the elderly showing signs of some diseases such as Alzheimer's, atherosclerosis, cancer, inflammation and Type 2 diabetes (Stadtman 2006). In these circumstances, the intake of supplements or consumption of foods rich in antioxidants could help to minimise such adverse reactions. Regarding dairy products, some casein and whey proteins and their short peptides from their hydrolysates could maintain the correct balance.

Anti-inflammatory effect

Inflammation, whether acute or chronic, has been recognised as the basis of pathogenesis of many diseases such as Alzheimer's, cancer, cardiovascular, diabetes and osteoporosis. It has also been shown that symptoms as diverse as depression, muscle fatigue, tiredness and

few more might also be linked, to some degree, to inflammation. Although inflammation is a defensive mechanism against foreign agents entering the body or as a result of internal injury, this action needs to be controlled before causing further damage to the tissues.

The majority of people tend to use the general over-the-counter medication based on non-steroidal anti-inflammatory drugs (NSAID). However, some people rely on diet and/or nutritional supplements as a means to minimise inflammation rather than taking medication. The best-known supplement is the omega-3 fatty acids such as docosahexaenoic acid (DHA) or conjugated linoleic acid (CLA), especially in inflammatory bowel disease (Simopoulos 2002). Other supplements which been demonstrated to have an effect on inflammation include: vitamins such as A, C, D and E; minerals such as Zn, Se and Cr; fruits, vegetables and their extracts such as tomato lycopene; probiotics; and some well-known peptides.

Milk proteins and peptides have been used *in vivo* and *in vitro* to assess their effect as anti-inflammatory agents. The non-heme and iron-binding glycoprotein lactoferrin, along with immunoglobulins, are vital protective proteins in an innate immune mechanism. The role of lactoferrin in regulating and controlling the production of cytokines such as tumour necrosis factor alpha (TNF- α) and interleukin-1 β , IL-1 β and IL-6 (Crouch *et al.* 1992; Conneely 2001) has also been demonstrated. Glycomacropptides, fraction 106–169 from κ -casein, and other casein tryptic hydrolysates and proteose peptone-3 also contribute to anti-inflammatory action. There are also some commercially available synthetic anti-inflammatory oligopeptides of about 7–9 amino acids with molecular weight around 1 kDa. Most of these rely on a specific sequence of a few amino acids in a certain position on the peptide chain.

Immunomodulation

The immune system protects the body from infective and foreign agents by destroying them through an effective mechanism based on immunoglobulins and other agents. It was found that certain milk proteins and their hydrolysates could be effective immunomodulators. For example, lactoferrin and whey protein hydrolysate enhances the production of immunoglobulins IgA, IgM and IgG (Miyachi *et al.* 1997). Similarly, other whey proteins such as α -lactalbumin and β -lactoglobulin hydrolysates containing peptide fractions with 2–10 amino acids could enhance activity and lymphocyte proliferation (Biziulevicius *et al.* 2006; Jacquot *et al.* 2010). The effect of partially hydrolysed β -Casein, fraction 1–28, on the immune modulation was examined for its effect on human T, B and monocytes cell lines proliferation. The study showed that the proliferation of these cell lines was improved. It was also shown that after 96 hours of incubation, IgA production was stimulated (Kawahara *et al.* 2004). In an *in vivo* study, Otani *et al.* (2000) demonstrated that oral administration of diets containing fractions extracted from caseinophosphopeptides, CPP-I hydrolysates such as fraction 1–32 from α -casein and 1–28 from β -casein, enhanced intestinal IgA levels in animals. Similarly, Kitamura & Otani (2002) showed that oral administration of a diet containing CPP-III enhanced fecal IgA level in healthy human subjects.

Most researchers have shown that the physiological effects of the specific peptide fractions are usually associated with a known chain length and amino acid sequence. However, it is interesting to examine the synergistic effect of a mixture of several peptide fractions which could yield better results than the individual fractions.

1.6.2 Other bioactivities

Several other bioactivities related to protein and peptides which affect human health are briefly discussed in the following sections.

Ageing

The proportion of the ageing population is increasing due to better health care, nutrition and the improved understanding of the importance of a balanced diet to enhance health. The process of ageing within the human body is still not yet fully understood, and is usually linked to the gradual diminishing of the physiological function of the cell as well as genetics. However, it is still linked to the presence of reactive oxygen species (ROS) and their effect on cellular function which could lead to their malfunction and the manifestation of several diseases such as Alzheimer's, arthritis, cancer, diabetes and Parkinson's. Ageing has been linked to many other factors, one of which is metabolic rate as well as the effect of oxidative stress and lack of antioxidants. It seems clear that taking some supplements such as vitamins, minerals, omega-3, antioxidants, etc. may, to some degree, reduce the rate of ageing. Other factors such as exercise, nutrition and some social factors also have an impact on ageing.

Much research has been published regarding the process of ageing, linked to the shortening of the telomeres which could be limited by the presence of carnosine, a dipeptide of β -alanine and histidine (Nagasawa *et al.* 2001). Some even demonstrated a rejuvenating effect in the cell. More research regarding the assessment and severity of ageing has focused on biomarkers such as telomerase deficiency or overexpression and its link to telomere length homeostasis and cell viability (Bianchi & Shore 2008).

Opioids and satiety

Opioids are bioactive molecules which bind to opioid receptors in the nervous system and gastrointestinal tract and can affect mood, emotion and behaviour. They might have an indirect effect on food intake by inducing satiety. Many opioids can pass through the epithelial membranes of the gastrointestinal tract to the bloodstream and reach the brain and the central nervous system. Peptide-based opioids such as casomorphins are released after enzymatic hydrolysis of the casein. Gauthier & Pouliot (2003) have found that β -lg releases a hydrophobic opioid tetra peptide, fraction 102–105, which could also undergo further interaction with the central cavity of β -lg. Interestingly, β -lg interaction with peptide could also be used as a carrier to deliver peptides which might undergo further hydrolysis by gastric enzymes. Other opioid peptides from protein hydrolysis have been described by Meisel (1996) and Teschemacher *et al.* (1997).

Anticarcinogenic peptides

It appears that the anticarcinogenic properties of physiologically active compounds are mainly related to their indirect effect of enhancing the immune system and, in a few cases, direct effect in shrinking the tumour. Some of these bioactive compounds have been linked to the intact protein such as the role of lactoferrin from whey; it is thought that its binding to the iron in the tissue and reducing its oxidative role has an effect on oral carcinoma cells

(Mohan *et al.* 2006). It has also been found that some synthesised peptides containing 21 and 26 amino acids with two functional domains could facilitate the internalisation of these peptides into the targeted cancerous cells to induce apoptosis (Ellerby *et al.* 1999).

Wound healing

There are many bioactive components in food which could enhance wound healing. Some of these studies have been based on using the entire or whole food such as honey, and observing the rate of wound recovery. Milk protein and especially peptides derived from whey protein have also been shown to have a similar effect in wound healing. In a study by Wang *et al.* (2010), they found that intragastric administration of whey protein to rats improved wound healing compared to the control.

Antimicrobial properties

Some of the naturally released peptides by the gastrointestinal mucosa or the epithelial cells of the intestine, including defensins, bind to the negatively charged phospholipids on the microbial cell membrane and cause their destruction (Ramasundara *et al.* 2009). However, there is a fine balance between these and the probiotics present in the GIT (Jager *et al.* 2010). As the resistance of some bacteria to antimicrobial agents increases, including those based on naturally produced peptides, the emphasis is now on the synthesis of some peptides as anti-infective agents.

1.7 Probiotics and dairy products

The human gut contains a huge number (estimated to be over 100 billion) of micro-organisms, referred to as microbiota/microflora. These micro-organisms are normally divided into three categories: the beneficial, termed as probiotic bacteria; the harmful pathogenic; and the commensal bacteria with no harmful effect. The balance is naturally kept under control in the human gut in healthy individuals. During illness and infection however, as in the case of diarrhoea, the balance is tipped where the number of the harmful bacteria exceeds those of the beneficial bacteria. The use of antibiotics is then necessary to halt the effect of the harmful bacteria to restore the balance. It has been recognised however that the use of the antibiotic itself can inflict diarrhoea unrelated to pathogens, referred to as ‘antibiotic-associated diarrhoea’ (Hampel *et al.* 2012). Some of the probiotics such as *Lactobacillus rhamnosus* GG, *Lb acidophilus* and *Lb reuteri* have been used in the case of travellers or antibiotics-related diarrhoea (Katelaris *et al.* 1995; Vanderhoof *et al.* 1999).

The main probiotic bacteria that have been studied and used in fermented dairy products and other foods are members of *Lactobacillus* spp and *Bifidobacterium* spp (Sanders 2000). To derive their health benefits, it was suggested they should be present in these products at concentrations of more than 10^6 cfu g^{-1} or 10^6 cfu mL^{-1} of a product (Shah 2000). There are several factors that affect the viability and survival of the probiotics, some of which have been attributed to the storage temperature, pH and the type of acids added or produced such

as acetic acid or ascorbic acid and the level of oxygen (Dave & Shah 1997; Kailasapathy & Chin 2000).

The beneficial effects of probiotics taken in fermented foods materialise after their ingestion and survival within the gastrointestinal tract of the host, where they colonise the GIT and prevent the adherence of the pathogenic bacteria. They can also have an indirect effect through the production of some bioactive compounds such as antimicrobial and immunomodulating agents as described in Section 1.6.1. The antimicrobial agents can be isolated and applied to many dairy products, such as cheese, to improve their shelf life.

Fermented dairy products such as drinking yogurt has dominated the probiotic market and been regarded as the main source of probiotic intake (Tamime *et al.* 1995). Their inclusion into other products has been limited to a few other dairy products, for example, ice-cream and cheese. Micro-encapsulation technology has made their use in more varied products possible, where some heat processing or unfavourable conditions exist for example (Shah & Ravula 2000).

1.8 Summary and future trends

Milk and dairy products are an essential part of a healthy diet. They have nutritional as well as physiological functions which need to be explored further to improve human health. In the past few decades there has been considerable progress in the understanding of many biochemical and analytical techniques which has enabled researchers to better interpret the research data. Advances in bioinformatics and the emergence of ‘omics’ technology has created a huge interest in the field; progress in genomics, proteomics and peptidomics has created many discoveries that could link all these technologies together to understand the role of food or its bioactive components to improve human health. Computer-based docking has been used for the discovery of new interactions such as in the creation of novel peptides with specific amino acid sequences to perform certain physiological functions, which could be confirmed experimentally. More research should be undertaken to verify and confirm the physiological functions in human intervention trials, important to minimise the use of drugs (especially those with unpleasant side-effects). Further research is also needed on the role of bioactive compounds, especially the bioactive peptides, and how to transfer the technology to produce supplements or new products for the consumer.

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